Differentiation of *Capsicum* species via protein extraction and SDS-PAGE analysis: A two-day experiment for undergraduate biochemistry laboratories

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Introduction

The *Capsicum* genus is comprised of more than 200 varieties of pepper plants that collectively represent only five cultivated species.[1] As a result of extensive cross-breeding, an 88% similarity in protein coding DNA has developed.[2,3] While this has made species determination based solely on physical appearance all but impossible[4], the 12% difference in protein coding DNA has led to subtle differences in translated protein.

The two main objectives for this research were:

1. To develop methodology to distinguish among five different species of pepper plants based on the proteins produced by each species, and
2. To use the methodology to develop a two day laboratory experience for biochemistry students.

The educational aspect of this project was successfully completed in two laboratory periods which allowed the students to use a variety of biochemical techniques (such as centrifugation, vacuum filtration, and SDS-PAGE) as well as more specialized procedures including natural product extraction, tissue preparation, and the handling of liquid nitrogen.

Methods

An extraction protocol from the Proteomics Center[5] was modified to create a method that uses common laboratory equipment, safer chemical components, and more comprehensible directions. Common biochemical techniques including centrifugation and vacuum filtration were used along with liquid nitrogen and acetone precipitation. The Extraction Media consists of 0.175 M Tris-HCl pH 8.8, 5% SDS, 15% glycerol, and 0.3 M DTT. The final Extraction Solution contains 8 M urea, 2M β-mercaptoethanol, 2% CHAPS, 2% Triton X-100, 50 mM DTT, and 0.5% pH 3-10 ampholytes. The banding patterns were created via SDS-PAGE analysis according to standard laboratory procedures.

SDS-PAGE:

1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis
2. A common technique used in the field of biochemistry that separates proteins based on size.
3. Uses a stain so proteins are visualized as bands.

Methodology

Day One:

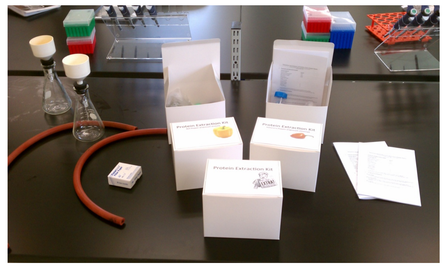
1. Crush 1 gram of seed to a fine powder with liquid nitrogen using a large mortar and pestle.
2. Mix powder with 5 mL of Extraction Media specifically developed for this research.
3. Filter homogenate via vacuum filtration and discard the solid waste.
4. Immediately add ice-cold acetone and store at -20 °C overnight to precipitate proteins.

Day Two:

1. Centrifuge at 5000 g for 15 min to collect precipitated protein. Wash pellet with acetone.
2. Repeat centrifugation and acetone wash.
3. Resuspend pellet in 1 mL of Extraction Solution and incubate at room temperature for one hour.
4. Centrifuge at 5000 g for 10 min to collect proteins in supernatant.
5. Analyze the protein samples via SDS-PAGE.
6. Stain with Coomassie blue.
7. Observe and analyze the resulting protein banding patterns.

Materials

A



B

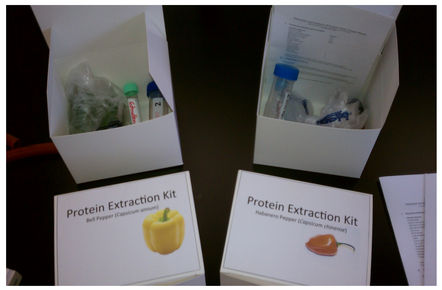


Figure 1. (A) Necessary lab materials include vacuum filtration apparatuses, custom lab kits, and instruction booklets for each group. (B) Kits were specially prepared containing fresh peppers and pre-measured amounts of each required solution.

Protein Banding Patterns

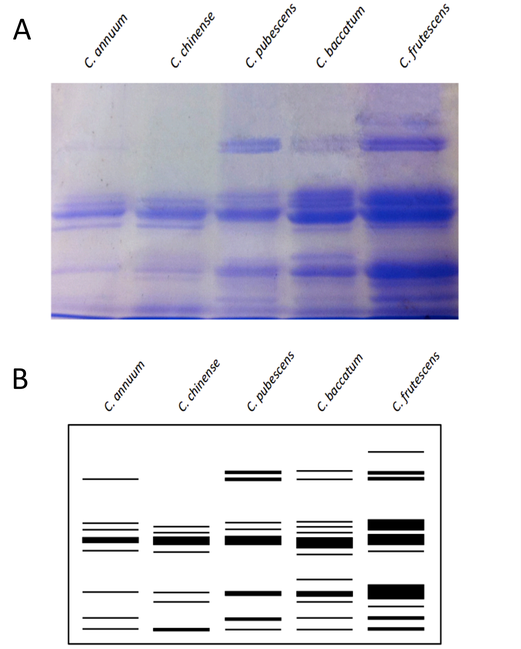


Figure 2. (A) Protein banding patterns for each cultivated *Capsicum* species were obtained via SDS-PAGE analysis. (B) Graphic representation of protein banding patterns to facilitate easy interpretation.

Phylogenetic Relationship

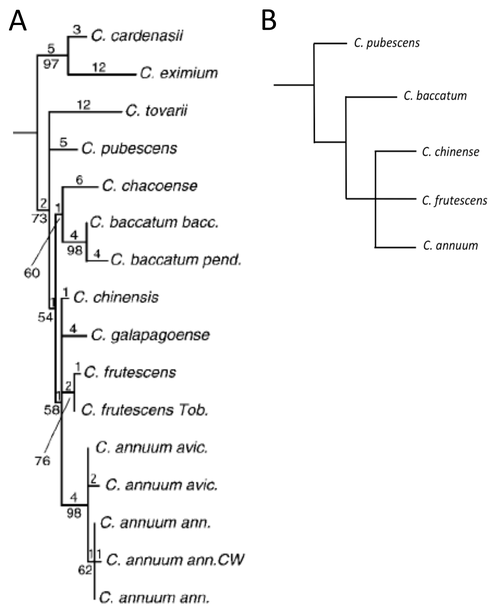


Figure 3. (A) Phylogenetic analysis of *Capsicum* genus based on combined atpB-rbcL spacer and waxy data.[6] (B) Simplified version of analysis by Walsh and Hoot that emphasizes the five species analyzed in this experiment.

Results/Conclusion

1. Proteins were successfully extracted from pepper plant seeds using the methodology that was developed.
2. SDS-PAGE analysis demonstrated clear differences in protein expression in the five variety of pepper plants as shown in figure B
3. An excellent laboratory was developed for use in the undergraduate biochemistry laboratory.

Extensions

The scope of this experiment can be increased with the application of potential extensions including:

1. Silver staining to reveal addition protein bands for further analysis.
2. Studies to determine if the differences in banding patterns are due to species-specific variation or simply different varieties.
3. Bradford assay to standardize the amount of protein to allow comparative analysis.

Acknowledgements

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